# A rapid method of sectioning dried coniferous needles for mycological studies

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A method is described whereby thin sections of dry herbarium coniferous needles may be cut on a cryostat microtome. Stained, temporary slides suitable for diagnostic and (or) photomicrography purposes may be made in 2½ hours. Permanent slides may also be made by this method.

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Studies of the taxonomy and infection processes of fungal parasites affecting coniferous needles sometimes require the examination and comparison of sections from many different specimens. In many instances, the needles are available only in a dried condition. Sectioning of this material is impossible without some preconditioning of the tissue.

The usual method of boiling dried specimens in distilled water to soften them and of cutting sections with a hand microtome, or by holding the material in pith and sectioning with a razor, is not entirely satisfactory. Even in experienced hands, sections obtained are frequently variable in thickness and torn or otherwise distorted by the time they are mounted. The use of the paraffin method of infiltration and embedding before sectioning is often unsatisfactory because of distortion and the lengthy processing period.

A method that overcomes the objectionable aspects of the usual procedures was brought to my attention by Dr. James Reid of the Botany Department of the University of Manitoba. Dr. Reid and others have used this process for some time in preparing temporary slides from a wide variety of mycological specimens. Briefly, it consists of soaking segments of the specimens in distilled water for ½ to 1 hour; infiltrating them in a solution of 1:1 water and Carter's mucilage;* sectioning with a freezing microtome or a cryostat; rinsing sections in distilled water and then mounting in lactophenol – cotton blue or lactophenol – acid fuchsin. The purpose of this note is to describe the method in detail and describe an adaptation for the preparation of permanent mounts. The following procedure has been used with excellent results to examine dried needles of Douglas fir, *Pseudotsuga menziesii* (Mirb.) Franco, infected with a fungus, *Rhabdocline* species (Fig. 1).

1. Cut dried needle into desired lengths.
2. Place tissue in distilled water to which a few drops of "Aerosol OT solution"† have been added, for ½ to 1 hour.
3. Infiltrate needle pieces for 1 hour in a solution of 1:1 water and Lepage's mucilage.‡ A longer period of infiltration does not seem to be deleterious to the host tissue or the parasite.
4. Mount infiltrated tissue in the same solution on a microtome chuck and freeze on cold bar of a cryostat at a temperature of −15 C.
5. Cut sections at 10 to 15 μ.
6. Rinse sections gently in distilled water for 10–15 minutes to remove mucilage from tissue.
7. Float sections on a drop of water on a glass slide and place in oven at 56 C for a few moments to evaporate water from slide.
8. Mount in lactophenol – cotton blue or lactophenol – acid fuchsin (Sass 1951).

Permanent, stained mounts may be made by following steps 1 to 6 inclusive and then carrying out the additional steps outlined below.

1. Coat a slide lightly with Haupt's adhesive.
2. Float sections on a drop of 4% formalin and place in an oven at 56 C filled with formalin fumes for about ½ hour to harden the adhesive.
3. Immerse slide in absolute alcohol for 5 minutes.
4. Dip slide in 2:1:1 "Stanvis" Nicoloidine solution,§ and absolute alcohol – ether and allow to air-dry and place in 70% alcohol for 2 minutes and take through alcohols to water.

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*The Carter Ink Co. of Canada Ltd., Montreal, Quebec.

†Fisher Scientific, Vancouver, British Columbia.

‡Lepage's Ltd., Toronto, Ontario.

§British Drug House (Canada) Ltd., Vancouver, B.C.

5. Proceed with staining (I used the periodic acid – Schiff (P.A.S.) method described by Farris (1966)).

The method is simple and rapid, the resulting sections being uniform in thickness and free from tearing and distortion. A stained temporary slide, suitable for diagnostic and (or) photomicrography purposes can be made from a dry needle in about 2½ hours. Permanent slides can be made in about the same time, with additional time for staining, depending on the staining schedule used.

Some modification was found necessary when examining dried needles of alpine fir, *Abies lasiocarpa* (Hook.) Nutt., infected with a hypodermataceous fungus. I found that needles with early symptoms of disease could be processed as satisfactorily as the Douglas-fir needles, provided the period of soaking in distilled water was extended to 12 hours or overnight and the cutting temperature was raised to −10 C. Weaver and Layne (1965) also found that the optimum temperature for cutting, using a microtome cryostat, varied with different types of botanical material. Permanent slides, stained by the safranin and fast green method of Johansen (1940), were made of this material (Fig. 2). Johansen's method was slightly modified by reducing the picric acid differentiation fluid from 0.5% to 0.125% to allow better control of the safranin differentiation.

A further modification of the method was required when preparing temporary mounts of needles showing advanced symptoms of infection by the hypodermataceous fungus. Satisfactory mounts were made in the following manner.

1. Soak, infiltrate, freeze, and section as previously described.
2. Remove sections from microtome knife with a cold dissecting needle and place on a precooled slide in the cryostat.
3. Remove the slide with sections from the cold atmosphere of the cryostat and allow sections and mucilage to dry at room temperature for about 2 minutes. Placing a finger on the underside of the slide directly beneath the section or sections facilitates rapid drying.
4. Mount sections in a drop of lactophenol – cotton blue (Fig. 3).

When first mounted and viewed under a high power microscope the sections harbor copious numbers of small bubbles of mucilage which generally disappear in a few hours.

I thank Dr. James Reid of the University of Manitoba for bringing the basic technique to my attention. Dr. A. K. Parker and Dr. W. G. Ziller of this laboratory kindly supplied the needle material and offered helpful criticism of the manuscript. I also thank Mr. A. Craigmyle for his photographic assistance.

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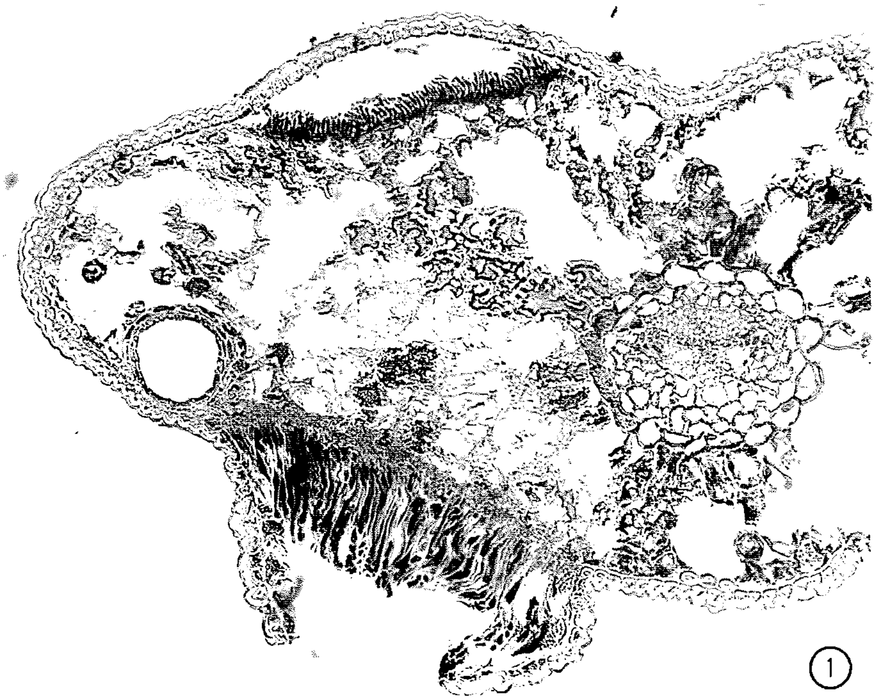
PLATE I



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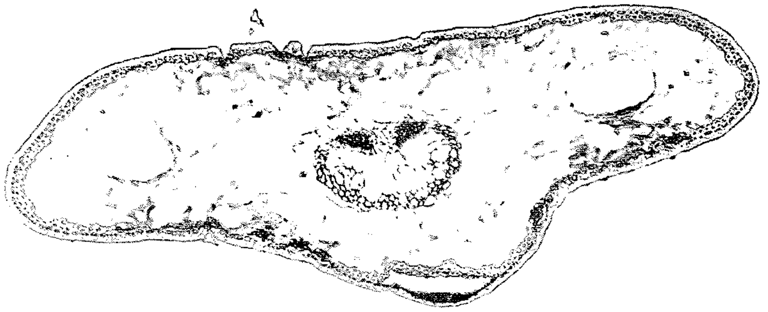

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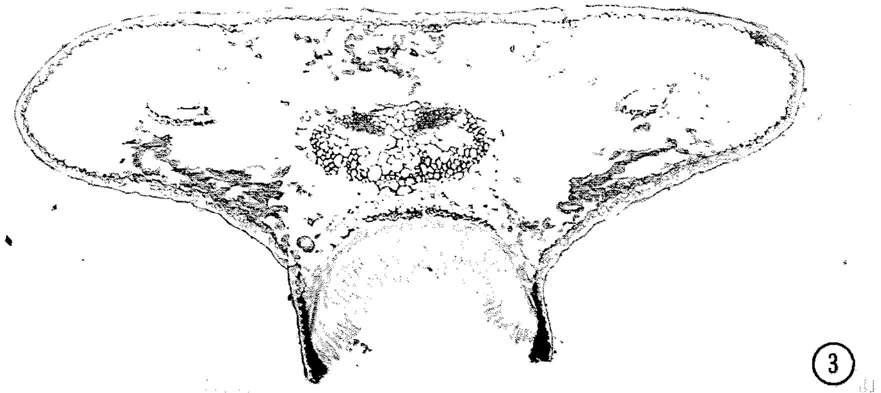

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FIG. 1. Cryostat section of Douglas-fir needle showing *Rhabdocline* on lower surface and *Rhabdogloeum pseudotsuga* on upper surface. × 160. Temporary mount in lactophenol – cotton blue.

FIG. 2. Cryostat section of *Abies* needle showing early symptoms of hypodermataceous infection. × 64. Permanent mount stained with safranin and fast green.

FIG. 3. Cryostat section of *Abies* needle showing advanced symptoms of hypodermataceous infection. × 64. Temporary mount in lactophenol – cotton blue.